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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant: Paul R. Schimmel

Serial No.: 08/249,689 Art Unit: 1631

Filed: May 26, 1994 Examiner: John S. Brusca

For: *"DESIGNING COMPOUNDS SPECIFICALLY INHIBITING RIBONUCLEIC ACID FUNCTION"*

Assistant Commissioner for Patents
Washington, D.C. 20231

APPEAL BRIEF

Sir:

This is an appeal from the rejection of claims 11-13, 17-19, and 21 in the Office Action mailed January 11, 2002, in the above-identified patent application, and maintained in the Advisory Action mailed June 10, 2002. The Appellant mailed a Notice of Appeal on June 10, 2002. A check in the amount of $215.00 for the filing of this Appeal Brief with a one month extension of time for a small entity is enclosed. It is believed that no additional fee is required with this submission. However, should an additional fee be required, the Commissioner is hereby authorized to charge the fee to Deposit Account No. 50-1868.

(1) REAL PARTY IN INTEREST

The real parties in interest of this application are the assignee, Massachusetts Institute of Technology, and licensee, Ariba Pharmaceuticals, San Diego, CA.

(2) RELATED APPEALS AND INTERFERENCES

This application has previously been on appeal, Appeal No. 1997-2396. There are no other related appeals or interferences known to appellant, the undersigned, or appellant's assignee which directly affects, which would be directly affected by, or which would have a bearing on the Board's decision in this appeal.

(3) STATUS OF CLAIMS ON APPEAL

Claims 1 and 3-21 are pending. Claims 1, 3-10, 14-16, and 20 have been allowed. Claims 11-13, 17-19, and 21 are on appeal. The text of each claim on appeal, as pending, is set forth in an Appendix to this Appeal Brief.

(4) STATUS OF AMENDMENTS

The claims were amended in an Amendment mailed July 2, 2001.

(5) SUMMARY OF THE INVENTION

The invention is based on the discovery that, unlike the case with DNA, the critical sites for targeting of an RNA molecule lie within the minor groove, where the compound binds by covalent or hydrogen binding to the secondary and tertiary structure of the RNA (page 5, 2-4). The claims are directed to a method for designing a compound that specifically inhibits the function of a targeted ribonucleic acid (claims 1, 3-10, 14-16, and 20, allowed), and the compound identified by the method (claims 11-13, 17-19 and 21, on appeal). The critical site of the targeted RNA is determined by a method such as mutational analysis, for example, site-directed mutational analysis (pages 9-18). The compounds are designed first by determining the

nucleotide of the critical site of the targeted ribonucleic acid (pages 9-18), then determining the secondary structure of the critical site of the targeted ribonucleic (pages 7-8), then determining the three-dimensional structure of the critical site of the targeted RNA, including the position of the critical site relative to the major and minor grooves (pages 37-38). The three dimensional structure of the critical site within target RNA is modeled using RNA sequence analysis and calculating the minimal energies for these structures (pages 37 and 18; claim 6 as originally filed). Appropriate molecules specifically inhibiting the function of the targeted RNA are synthesized using known methodology that have the required secondary structure and chemical characteristics (page 5, lines 10-13).

The claimed inhibitory compounds bind to the critical site within the minor groove, thereby inhibiting the targeted ribonucleic acid function (pages 38-39). The claimed compounds are defined by their function and by their structure, since they must be complementary to the critical site within the RNA to be inhibited. The compound is directed to and binds to a critical region of the RNA molecule, located with the minor groove of the RNA molecule, identified by a combination of the primary, secondary and tertiary structure of the critical region (pages 7 and 8). The compound contains hydrogen bond donor and acceptor sites arranged to specifically bind and inhibit the function of thetargeted RNA molecule (page 38, lines 24-31; and lines 29-17 bridging pages 38 and 39). The example in the application demonstrating reduction to practice utilized as the critical site the G3:U70 base pair (pages 31-33; page 36) within the minor groove of the acceptor stem of $tRNA^{Ala}$.

The target ribonucleic acid may be an mRNA, rRNA, tRNA, or a viral RNA (claim 3 as originally filed). The inhibitor can inhibit RNA function or protein synthesis *in vitro* or *in vivo* in a cell. Cells harboring the ribonucleic acid function to be inhibited may be tumor cells, virally infected cells, or bacterial cells (claim 5 as originally filed). The designed compound may be combined with a pharmaceutical carrier, for example, for topical administration, parenteral administration, or enteral administration (pages 39-41).

(6) ISSUES ON APPEAL

The issue presented on appeal is:

whether claims 11-13, 17-19, and 21 contain subject matter which was described in the specification in such a way as to reasonably convey that the inventor had possession of the claimed invention at the time the application was filed, under 35 U.S.C. §112, .

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. The claims can be grouped as follows: (1) claim 11, (2) claims 12, 18, and 19, and (3) claims 13, 17, and 21. Claim 11 is directed to a complementary compound harboring hydrogen bond donor and acceptor sites arranged to specifically bind and inhibit the function of a targeted RNA molecule, wherein the compound binds to a critical region of the targeted RNA molecule located within the minor groove of the minor groove. Claims 12, 18, and 19 are directed to further defining the targeted RNA. Claims 13, 17, and 21 are directed to further defining the complementary compound. Reasons for this grouping and arguments for the separate patentability of these groups of claims are provided below.

(8) ARGUMENTS

(a) The Claimed Invention

Appellant has made a pioneering invention - he has discovered the critical portion of an RNA molecule necessary for the design and synthesis of inhibitors that selectively bind to an RNA. This discovery can be analogized to that of an antibody, where the inhibitor is like an antigen and the minor groove of the RNA is like that of the antigen-specific binding sites of the antibody. One does not need to provide the exact nucleotide sequence encoding an antibody, or the exact structure of an antibody, in order to know what it is and how to use it. This is because the structure and function of an antibody is so well known. The same was true for the structure and function of an RNA molecule at the time this application was filed. The structure and chemical composition of RNA had been determined decades earlier, along with that of DNA. Both are formed of four randomly repeating nucleoside units, linked together to form a chain of unique sequence (referred to as "secondary structure"), which in turn forms a three dimensional structure (referred to as "tertiary structure"). However, while it was known at the time this application was filed that it was the major groove of the DNA that was critical for binding and function of the DNA, it was not known whether or not the major or the minor or both grooves were important for design of molecules that could specifically inhibit the function of the RNA.

RNA is an important molecule. It is critical to protein expression, serving as the messenger for the sequence to be expressed (mRNA), from the gene to the parts of the cells where proteins are actually made. It is the major form of genetic material in many viruses. It is also critical in protein expression, carrying or "transferring" the amino acids to be linked together

to form the proteins. The discovery that the minor groove is the most important structure of RNA in design of inhibitors has completely altered the way drugs are designed to be targeted to RNA.

The claims in issue were originally rejected under 35 U.S.C. §112 as non-enabled. The Board of Appeals determined that the claims were enabled, based on the record before them, but remanded on the issue of written description with respect to the claims drawn to the compounds used in the claimed method. The method claims are allowed.

The claims on appeal are drawn to a genus of compounds complementary to a targeted RNA molecule and inhibiting the function of the targeted RNA molecule. The basis of the is that appellant has failed to comply with the written description requirement defined by 35 U.S.C. 112. The basis for the rejection is that while appellant has provided evidence as to the functional aspects of the claimed compounds, he has not established a correlation between the required functions and the structures of the claimed molecules. This rejection is simply unfounded.

The specification describes the structure of the claimed compounds by illustrating the chemical properties (hydrogen bond acceptor and donor sites arranged specifically) and method of preparation (first, determining the target RNA sequence and second, preparing the compounds accordingly) of the compounds. These features have been incorporated into the claims. These elements distinguish the compounds based on the claimed interaction with a critical region in the minor groove of the target RNA. Although the compounds may be organic, inorganic, proteins, or even nucleic acids, specific binding is achieved through complementary interactions (page 38 of the specification, lines 24-31). These interactions are dependent upon hydrogen bonding

(lines 29-17, bridging pages 38 and 39). Therefore, in order for the compound to bind to the target RNA, hydrogen bond donor sites, hydrogen bond acceptor sites, and chemical side groups, have to be in the correct spatial location, orientation, and have the correct charge. One of skill in the art would realize that it is this arrangement that defines the structure of the compound. "Complementary" defines the structure of the compound. Complementary compounds are limited by the sequence of the RNA target molecule. Given the minor groove sequence of the RNA to be targeted, the arrangement of possible hydrogen bonds to be utilized by the compound is defined, therefore limiting the structure of the compound.

As stated in M.P.E.P. § 2173.05(t), which describes the standard to be applied to compounds and compositions, "a compound of unknown structure may be claimed by a combination of physical and chemical characteristics." See *Ex parte Brian*, 118 USPQ 242 (Bd. App. 1958). M.P.E.P. § 2173.05(t) further states that "a compound may also be claimed in terms of the process by which it is made without raising an issue of indefiniteness." It is important to note that only *after* obtaining the correct target RNA sequence, can the claimed compound and its structure be elucidated. This, however, is routine to those skilled in the art. The structural features common to the members of the claimed genus can only be determined once the hydrogen bonding arrangement of the target sequence is derived. Once the RNA sequence is derived, the minor groove structure can be easily inserted into any number of commercially available computer programs and the structural features of the compound determined. The structure of the compound is clearly limited based on the requirement for it to be complementary to the RNA sequence.

The complementary nature of the compound of independent claim 11 distinguishes the claimed compound from others. Compounds that bind a RNA in the minor groove would not necessarily have the requisite correct charge and spatial orientation of the potential hydrogen bond donors and acceptors to be specific for presentation and binding to the targeted critical region of a RNA molecule. While most, if not all, compounds that bind RNA do have hydrogen bonding sites, only a few will have the necessary pattern of sites to be utilized specifically by the targeted critical region. The identification of the critical region within the minor groove by a combination of primary, secondary and tertiary structure analysis, as recited in claim 11, is required for the determination of the structure of the compound. Therefore the structural features common to the claimed compound, as defined by the term "complementary" and by containing the requisite hydrogen bonding acceptor and donor sites, are clearly described.

The specification also discloses other relevant information and identifying characteristics sufficient to describe the claimed invention. One of skill in the art would be able to predict the structure of the claimed complementary compound from the recitation of its function. In order for a compound to have a structure that is complementary to a targeted nucleic acid, hydrogen bond donor and acceptor sites must be properly arranged to bind the targeted molecule. To this end, as will be described below, the critical region of the targeted RNA molecule defines and limits the structure of the claimed genus of compounds.

Moreover, the relationship between these features of the claimed compounds, as well as their function, completely detail to those skilled in the art that appellant possessed the claimed

genus at the time of filing this application, and enabled those skilled in the art to know what does, and does not, fall within the scope of the claims.

The importance of appellant's discovery cannot be underestimated: he discovered that RNA inhibitors must bind to their RNA target within the minor groove, and can be designed based on the secondary and tertiary structure of the RNA within this minor groove. Many others have since made RNA inhibitors that bind to the minor groove. Appellant submitted Declarations by two experts in the field to show that they considered the application to comply with the written description requirement, and that one of routine skill would need nothing more than what is in the application to know that appellants had possession of the claimed genus at the time of filing. The Examiner in his advisory action indicated that the declarations were somehow deficient because they did not point to a particular part of the specification where the structure of the RNA was specified, but indeed, no expert would think such a description was necessary. Those skilled in the art at the time this application was filed knew the structure of RNA, including the minor groove. They also had available computer software programs that allowed one to enter the nucleotide sequence and thereby obtain the tertiary structure. What was missing was the evidence that the inhibitor had to fit within the minor groove, bound by hydrogen bonds and having complementary structure to the RNA within the minor groove. The examiner also seemed to doubt that such inhibitors had been made.

As noted above, the issue of enablement has been raised and overcome on appeal. However, the advisory action seems to raise again the issue of enablement, at least at whether such inhibitors can or could have been made. Although it is not believed to be an issue properly

on appeal, enclosed are representative abstracts showing that compounds that bind within the minor groove to inhibit RNA function, as claimed, have been made:

Clarke and Mathews, "Interactions between the double-stranded RNA binding motif and RNA: definition of the binding site for the interferon-induced protein kinase DAI (PKR) on adenovirus RNA 1(1):7-20 (1995)

Li, et al., "A heterocyclic inhibitor of the REV-RRE complex binds to RR as a dimer" Biochemistry 40(5):1150-1158 (2001)

With respect to the comment in the advisory action that the Williamson declaration shows a compound which is not an RNA inhibitor, enclosed are three abstracts that demonstrate that Dr. Williamson has made deletion mutants of the S15 molecule, which binds within the minor groove of its RNA binding partners as a critical step in assembly of the 30 S ribosomal subunit. At least some of these deletion mutants would be expected to prevent assembly of the 30 S ribosomal subunit by binding but not allowing subsequent assembly. See:

Agalarov, et al., "Structure of the S15,S6,S18-rRNA complex: assembly of the 30 ribosome central domain" Science 288(5463):107-113 (2000)

Recht and Williamson, "Central domain assembly: thermodynamics and kinetics of S6 and S18 binding to an S15-RNA complex" J. Mol. Biol. 313(1):34-48 (2001)

Scott and Williamson, "Interaction of the Bacillus stearothermophilus ribosomal protein S15 with its 5'-translational operator mRNA" J. Mol. Biol. 314(3):413-422 (2001).

Accordingly, although there are no examples of the design, synthesis and testing of RNA inhibitors that bind to the minor groove in the application as filed, those skilled in the art do not

believe such evidence is required in order to know that appellant had the claimed genus in his possession: i.e., that he had described the function and structure with sufficient detail to establish a correlation between the two.

(b) Rejections Under 35 U.S.C. § 112, First Paragraph (Written Description)

Claims 11-13, 17-19, and 21 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that (1) the application does not provide a working example of the structure of a representative number of species of the claimed genus of compounds; and that (2) appellant has not shown that there is an art-recognized correlation between structure and the claimed function.

(i) The Legal Standard for Written Description.

The first paragraph of 35 U.S.C. § 112 sets forth the written description requirement for patents as follows:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention."

The standard regarding what is or is not supported by the specification has been clearly articulated as "requiring the specification to convey with reasonable clarity to those skilled in the art that, as of the filing date sought, the inventor was in possession of the invention", i.e., whatever is now claimed. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111, 1117 (Fed . Cir. 1991). Compliance with the written description requirement is essentially a fact-

based inquiry that will "necessarily vary depending on the nature of the invention claimed." *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991) (citing *In re DiLeone*, 436 F.2d 1404, 1405 (CCPA 1971)). Satisfaction of the written description requirement is determined on a case-by-case basis.

The inquiry into whether or not there is an adequate written description is not performed in a vacuum. "Knowledge of one skilled in the art is relevant to meeting [the written description] requirement." *Enzo Biochem, Inc. v. Gen-Probe*, Docket No. 01-1230 (Fed. Cir. Apr. 2, 2002) (slip op.). This fact has implications not only for validity challenges, but also for patent prosecution. *See In re Alton*, 76 F.3d 1168, 1174-75 (Fed. Cir. 1996).

In the most recent CAFC decision, *Enzo Biochem, Inc. v. Gen-Probe*, Docket No. 01-1230 (Fed. Cir. July 15, 2002), the Federal Circuit vacated a prior decision, *Enzo Biochem, Inc. v. Gen-Probe*, 285 F.3d 1013, 62 USPQ 2d 1289 (Fed. Cir. April 2, 2002), and reversed the district court's grant of summary judgment that Enzo's claims are invalid for failure to meet the written description requirement, stating in relevant part:

"It is not correct, however, that all functional descriptions of genetic material fail to meet the written description requirement. The PTO has issued Guidelines governing its internal practice for addressing that issue. The Guidelines, like the Manual of Patent Examining Procedure ("MPEP"), are not binding on this court, but may be given judicial notice to the extent they do not conflict with the statute. See Molins PLC v. Textron, Inc., 48 F.3d 1172, 1180 n.10, 33 USPQ2d 1823, 1828 n.10 (Fed. Cir. 1995). In its Guidelines, the PTO has determined that the written description requirement can be met by "show[ing] that an invention is complete by

disclosure of sufficiently detailed, relevant identifying characteristics . . . *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics." Guidelines, 66 Fed. Reg. at 1106 (emphasis added). For example, the PTO would find compliance with § 112, ¶ 1, for a claim to an "isolated antibody capable of binding to antigen X," notwithstanding the functional definition of the antibody, in light of "the art-recognized method of making antibodies *to fully characterized antigens*, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the *antibody technology is well developed and mature*." (emphasis added) Synopsis of Application of Written Description Guidelines, at 60, available at http://www.uspto.gov/web/patents/guides.htm ("Application of Guidelines"). With reference to the claimed nucleotide sequences of Enzo, the Board also noted that "[B]ecause the claimed nucleotide sequences preferentially bind to the genomic DNA of the deposited strains of *N. gonorrhoeae* and have a complementary structural relationship with that DNA, those sequences, under the PTO Guidelines, may also be adequately described.....[A]lthough the patent specification lacks description of the location along the bacterial DNA to which the claimed sequences bind, Enzo has at least raised a genuine issue of material fact as to whether a reasonable fact-finder could conclude that *the claimed sequences are described by their ability to hybridize to structures that, while not explicitly sequenced, are accessible to the public*."

The PTO Guidelines clearly state that the written description requirement can be met by "showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying

characteristics....i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. Guidelines, 66 Fed. at 1106." (emphasis added) *Enzo Biochem, Inc. v. Gen-Probe*, Docket No. 01-1230 (Fed. Cir. July 15, 2002).

The general principle of the written description requirement for a claimed genus may be satisfied through (1) sufficient description of a representative number of species by actual reduction to practice, (2) reduction to drawings of a general structure, or (3) disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, (4) describing functional characteristics coupled with a known or disclosed correlation between function and structure, or (5) a combination of such identifying characteristics, sufficient to show the appellant was in possession of the claimed genus. Reagents of the University of California v. Eli Lilly, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

In summary, actual reduction to practice of a representative number of species is NOT required. In this case, appellant has functional characteristics coupled with a correlation between function and structure. Appellant also has expert testimony that the specification describes the claimed genus to one of ordinary skill in the art in such a way that they know what the claimed genus is, and thereby that the appellant had possession of the claimed genus at the time of filing. Still further, appellants have shown post filing reduction to practice of representative species of the claimed genus.

With regard to post-filing art, the CAFC stated in In re Brana, 51 F.3d 1560, 1567 n.19 (Fed. Cir. 1995), that a post-filing date declaration setting forth test results substantiating utility "pertains to the accuracy of a statement already in the specification. . . . It does not render an insufficient disclosure enabling, but instead goes to prove that the disclosure was in fact enabling when filed." An important distinction has been made by the Courts between evidence of the knowledge and ability of those of skill in the art at the time of filing and evidence to prove that statements made in the application are correct. In the former case, of course, only evidence which existed prior to the filing of the application, or evidence that certain knowledge existed at the time of filing, is admissible (In re Hogan, 194 USPQ 527 (CCPA 1977)). In the latter case, as in this case, any evidence, developed at any time, may be submitted for consideration.

The clearest affirmation of the seasonability of factual evidence developed after the filing date of an application is provided by the Court in In re Marzocchi (169 USPQ 367, 370 (CCPA 1971)). In discussing rejections under 35 USC 112 where an examiner asserts that the unpredictability of the art creates a reasonable doubt as to the accuracy of a particular broad statement (in the application) supporting enablement, the Court states:

> Most often, additional factors, such as the teachings of pertinent references[*], will be available to substantiate any doubts that the asserted scope of enablement is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof.
>
> ***

> Not necessarily *prior* art references, it should be noted, since the question would be regarding the *accuracy* of a statement in the specification, not whether that statement had been made before. [emphasis in the original]
>
> *Id. at 367*

In *In re Wilson* (135 USPQ 442, 444 (CCPA 1962)), the Court agreed that a reference, published after the filing date of the application, was properly cited to show a state of fact. In *In re Langer* (183 USPQ 288, 297 (CCPA 1974)), the Court again noted that later published references "are properly cited for the purpose of showing a fact." In *In re Rainer* (134 USPQ 343, 345 (CCPA 1962)) the Court found no error in the limited use made of a reference published after Appellant's filing date to show a fact. While all of these cases involved publications cited by the Patent Office in support of rejections, the same standard applies to evidence cited by Appellant. See In re Hogan.

It is not necessary, nor is it required, that each element of the claimed invention be within a single post filing art reference. It is the evidence as a whole that must be considered. Elements of the claimed invention *independently* described in the post filing art, can cumulatively demonstrate the feasibility of reducing the invention to practice using materials and methods described in the specification and/or known by a skilled artisan as of the time of filing.

Lastly, there is no legal requirement that an inventor have actually reduced the invention to practice prior to filing. MPEP at § 2164.02, *citing* Gould v. Quigg, 822 F.2d 1074 (Fed. Cir. 1987). "The specification need not contain an example if the invention is otherwise disclosed in

such a manner that one skilled in the art will be able to practice it without an undue amount of experimentation." *Id.*

(ii) The Claims Recite Structure and Function

The sufficiency of the specification and the expert opinion is discussed above, and in more detail below. The examiner has provided no evidence to rebut this expert opinion. The claims further define the claimed compounds in terms of both structure and function.

Claim 11 recites: A complementary compound comprising

hydrogen bond donor and acceptor sites arranged to specifically bind and inhibit the function of a targeted RNA molecule,

wherein the compound is specifically directed to and binds to a critical region of the RNA molecule, located within the minor groove of the RNA molecule, identified by a combination of the primary, secondary and tertiary structure of the critical region.

The inhibitory function of the claimed compounds cannot be obtained without a complementary structure as defined by the described chemical properties. The specification describes the structure of the claimed compounds by illustrating the chemical properties (hydrogen bond acceptor and donor sites arranged specifically) and method of preparation (first determining the target RNA sequence and second, preparing the compounds accordingly) of the compounds. These features are incorporated into the claims. These elements define the compounds based on the claimed interaction with a critical region in the minor groove of the target RNA. Although the compounds may be organic, inorganic, proteins, or even nucleic acids, specific binding is achieved through complementary interactions (page 38 of the

specification, lines 24-31). These interactions are dependent upon hydrogen bonding (lines 29-17, bridging pages 38 and 39). Therefore, in order for the compound to bind to the target RNA, hydrogen bond donor sites, hydrogen bond acceptor sites, and chemical side groups, have to be in the correct spatial location, orientation, and have the correct charge. One of skill in the art would realize that it is this arrangement that defines the structure of the compound.

This is in fact evidenced by the declarations submitted by appellants with the last response. Declarations under 37 C.F.R. § 1.132 by Dr. Jules Rebek and Dr. James R. Williamson, respectively, were submitted with the response mailed on April 11, 2002. Both, while also qualifying as experts, have opined that one of skill in the art would have a mental picture of the structure of the chemical, or be able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it, the standard articulated by the Federal Circuit in Amgen, 927 F.2d at 1206,18 USPQ 2d at 1021, as discussed below.

"Complementary" defines the structure of the compound since the compounds are limited by the sequence of the RNA target molecule. Given the minor groove sequence of the RNA to be targeted, the arrangement of possible hydrogen bonds to be utilized by the compound is defined, therefore limiting the structure of the compound. *This arrangement of hydrogen bonds is not descriptive of an inhibitory function, but rather is descriptive of the structure of the claimed compounds. The Examiner has severely misinterpreted the terms "function" and "structure" as depicted in the specification and claims. The claimed compounds do not function to form hydrogen bonds, as suggested by the Examiner (see page 2 of Advisory Action), but*

*rather function to inhibit (they **already** have the correct orientation of hydrogen bond donor and acceptor sites that allow them to access and bind in the targeted RNA minor groove).* The complementary compound of claim 11 comprises hydrogen bond donor and acceptor sites *necessary* to inhibit the function of a targeted RNA molecule. Hydrogen bond donor and acceptor sites are *structural elements* of the claimed compounds, without which, the claimed compounds could not properly inhibit the function of the targeted RNA. The hydrogen bond donor and acceptor sites define the landscape/topography of the claimed compound's surface (i.e. one-half of the interface between compound and targeted critical region).

(iii) USPTO Example For Applying Written Description Guidelines and Analogous Structure/Function Relationship

In the foregoing section, discussing the legal standard for written description, the appellant re-iterated the USPTO's clearly stated section for applying the written description guidelines, wherein "one of skill in the art would have recognized that the spectrum of antibodies which bind to antigens were implicitly disclosed *as a result of the isolation of antigen X.*" (emphasis added) Synopsis of Application of Written Description Guidelines, at 60, available at http://www.uspto.gov/web/patents/guides.htm ("Application of Guidelines"). The appellant respectfully submits that it is well recognized in the art that while antibodies retain the basic Y-shaped molecule structure, composed of two H (heavy) and two L (light) chains, they are differentiated based upon their antigen binding sites. Different binding sites are generated with different amino acid side chains in these positions, which are commonly known in the art as complementarity determining regions, or CDRs, and are located at the end of the variable light

and variable heavy chains. It is well established in the art that, based upon the structural features of antibody recognition of epitope, at least three forces drive antigen/epitope binding: 1) *hydrogen bonding* between donor-acceptor pairs on the variable regions and the targeted epitope; 2) water molecules that may be present at the antibody-antigen interface contribute to the complex *hydrogen bonding* pattern between molecules; and 3) numerous *van der Waals interactions*. These forces provide for the exquisite **complementarity** at the interface between the antibody and its targeted epitope. The appellant has consistently argued that these exact forces are what dictates the structure of the claimed compounds. Although the compounds may be organic, inorganic, proteins, or even nucleic acids, specific binding is achieved through **complementary** interactions (page 38 of the specification, lines 24-31). These interactions are dependent upon hydrogen bonding (lines 29-17, bridging pages 38 and 39). Therefore, in order for the compound to bind to the target RNA, hydrogen bond donor sites, hydrogen bond acceptor sites, and chemical side groups, have to be in the correct spatial location, orientation, and have the correct charge. The identification of the critical region within the minor groove by a combination of primary, secondary and tertiary structure analysis, as recited in claim 11, is required for the determination of the structure of the compound. This identification is parallel to the "isolation of antigen X", as stated in the example provided in the written description guidelines and quoted above. Therefore, not only has the targeted RNA been fully characterized (analogous to the isolation and characterization of antigen X), but the forces that drive the complementary interactions between antibody/antigen and compound/RNA *are the same*. These complementary interactions, as defined by the CDRs (**c**omplementarity **d**etermining **r**egions) of

antibodies, and the complementary region of the claimed compounds **define their respective structures** (i.e. the CDRs provide specificity to the staggeringly large repertoire of antibodies with different antigen-binding capabilities and are the basis for the immune system's ability to recognize virtually all foreign antigens) in view of their targeted epitope or RNA. It is no coincidence that the **antibody**/epitope and the claimed **compound**/substrate interactions and structures are defined using the same, well acknowledged and understood term in the art: ***"complementarity"***. Both, antibodies and nucleic acid hybridizing compounds are now designed based upon *known* epitope/antigen and nucleic acid. Once these "substrate" structures are known, complementary interactions lie at the core of producing a well defined structure that is able to recognize and bind to the target (i.e. like a "lock and key" – see below and accompanying declarations).

As stated in M.P.E.P. § 2173.05(t), which describes the standard to be applied to compounds and compositions, "a compound of unknown structure may be claimed by a combination of physical and chemical characteristics." See *Ex parte Brian*, 118 USPQ 242 (Bd. App. 1958). M.P.E.P. § 2173.05(t) further states that "a compound may also be claimed in terms of the process by which it is made without raising an issue of indefiniteness." It is important to note that only *after* obtaining the correct target RNA sequence, can the claimed compound and its structure be elucidated. This, however, is routine to those skilled in the art. The structural features common to the members of the claimed genus can only be determined once the hydrogen bonding arrangement of the target sequence is derived. Once the RNA sequence is derived, the minor groove structure can be easily inserted into any number of commercially

available computer programs and the structural features of the compound determined. The structure of the compound is clearly limited based on the requirement for it to be complementary to the RNA sequence.

The ease by which one can design and make compounds as defined by the claims is of course the reason the claims to the compounds are so important. Once one of skill in the art knows that one must target the minor groove of the RNA (rather than the major groove as is the case with DNA), then one has no difficulty in obtaining compounds. Indeed, this is shown clearly by the enclosed abstracts, as well as declarations by Dr. Williamson and Dr. Jules Rebek. As Dr. Williamson states, there was precedence for targeting to a groove of a nucleic acid helix, although it was not the minor groove, and many software programs were available that make it completely routine to insert the known nucleotide sequence of the target RNA into the program, and have it display *structures* that define the shape and composition of the claimed inhibitor (see pages 7-8 of the declaration).

The complementary nature of the compound defined by claim 11 distinguishes the claimed genus of compounds from others. Compounds that bind a RNA in the minor groove would not necessarily have the requisite correct charge and spatial orientation of the potential hydrogen bond donors and acceptors to be specific for presentation and binding to the targeted critical region of a RNA molecule. While most, if not all, compounds that bind RNA do have hydrogen bonding sites, only a few will have the necessary pattern of sites to be utilized specifically by the targeted critical region. The identification of the critical region within the minor groove by a combination of primary, secondary and tertiary structure analysis, as recited

in claim 11, is required for the determination of the structure of the compound. Therefore the structural features common to the claimed compound, as defined by the term "complementary" and by containing the requisite hydrogen bonding acceptor and donor sites, are clearly described.

The specification also discloses other relevant information and identifying characteristics sufficient to describe the claimed invention. One of skill in the art would be able to predict the structure of the claimed complementary compound from the recitation of its function. It is well established that the written description requirement may be satisfied through disclosure of function and minimal structure when there is a well-established correlation between structure and function. The appellant respectfully submits that this correlation has been established as described above. The hydrogen bonding pattern of the compound defines the structure of the compound, however, this defining characteristic is at the mercy of RNA analysis as described in base claim 11.

(iv) Those skilled in the art have testified the application meets the legal requirement for written description.

Declarations under 37 C.F.R. § 1.132 by Dr. Jules Rebek and Dr. James R. Williamson, respectively, were submitted with the response mailed on April 11, 2002. Both Dr. Rebek and Dr. Williamson are experts in the field. Dr. Williamson has provided his expert opinion as well as enclosed data in support of the claims. The declarations were submitted in order to provide further evidence that the description of the structure of the critical region in the minor groove of RNA is sufficient to describe the structure of the claimed compound. Each declaration clearly elaborates upon the present specification's discussion of the forces presented in and by the

targeted RNA molecule. While these forces establish the structure of the critical region of the RNA in terms of specific and available interactions and geometry, they are a direct result of the RNA sequence (primary structure). Secondary and tertiary structures can subsequently be determined via any number of commercially available programs, as outlined in the submitted declarations.

The analogy to a "lock and key", in the submitted declarations, is an important one because if one can conceptualize the role of the predetermined and defined target RNA in demanding a specific structure of the inhibitory compound, then one will realize that the compound structure is clearly defined. The target RNA is defined by those interactions and forces present in the minor groove of the critical region, as described in the specification, defined by the claims and further elaborated on by Drs. Jules Rebek and James R. Williamson.

Dr. Rebek

Dr. Rebek is the Director of the Skaggs Institute of Chemical Biology and Professor of Chemistry of the Scripps Research Institute. Dr. Rebek is clearly an expert in the field of nucleic acid structure. Dr. Rebek has no personal or financial interest in this application. He was asked to review the specification and claims, in view of the legal standard for the written description under 35 U.S.C. §112, to determine if he, as one in the field, would know what the structure of the claimed compounds was, based on his knowledge, the specification, and the language of the claims. Dr. Rebek specifically addressed the structure of the minor groove of the RNA in responding, reviewing the hydrophobic environment of the minor groove, hydrogen bonding, electrostatic interactions, and geometric and steric constraints. As summarized on page 7, "All

of these 'constraints' define the nature of the inhibitory compound in terms of structure and functionality; they define the molecular recognition of the RNA by the compound where the compound is complementary in size, shape and chemical surface to the RNA."

Dr. Williamson

Dr. Williamson is a Professor of Molecular Biology and Chemistry at the Scripps Research Institute in La Jolla, CA. He is an expert in the field of RNA and drug design, including RNA structure, RNA-protein recognition, and RNA-small molecule interaction. As stated at the top of page 3, he presents "evidence indicating that attractive and repulsive forces present in the critical region of the minor groove of RNA dictate or define the geometrical constraints of the region. These forces, as described in the specification, and below, define the structure of the critical region in a way that provides one with a mental picture of a defined "space" that can only be accessed by a compound of the correct "shape". He also reviews each of the claimed structural features: the hydrophobic environment of the minor groove, the hydrogen bonding, the electrostatic interactions, and the geometic and steric constraints. Dr. Williamson refers to the precedent of compounds that bind to DNA molecules (recognizing that here, the invention is the discovery that the minor groove of RNA is the critical binding site, whereas in DNA it is the major groove), as published by Dervan, et al., in Science 232, 464-471 (1986). Dr. Williamson also provides evidence that the claimed method and compounds are enabled and clearly described in view of his own subsequent work, published in part by Sultan, et al., Science 288, 107-112 (2000) and as demonstrated by the attached figures.

This evidence clearly support appellant's position that the specification and claims meet the requirements under 35 U.S.C. §112, written description.

(v) The Examiner has completely failed to individually examine the dependent claims.

It is well established that each claim must be separately examined for patentability. It is not enough, as here, to look at a single independent claim and reject all claims. No rationale has been presented as to why the claimed compounds may not further comprise a pharmaceutically acceptable carrier such as an acceptable composition for topical administration, an acceptable composition for parenteral administration, an acceptable composition for enteral administration, or combinations thereof, are not adequately described and fail to meet the standard under 35 U.S.C. § 112, first paragraph, for proper support based upon the written description. No rationale has been presented for why the written description describing the claimed compounds, wherein the compounds target are synthesized *in vivo* from a retroviral vector, does not meet the standard under U.S.C. § 112, first paragraph. Furthermore, no rationale has been presented as to why the written description does not adequately support the claimed compounds, wherein a critical region of a tRNA molecule is targeted (for example, the G3:U70 base pair of $tRNA^{Ala}$).

Claims 12, 18, and 19 are directed to further defining the targeted RNA. Claims 13, 17, and 21 are directed to further defining the complementary compound.

(vi) This is a Pioneering Invention

The claimed invention is an important and non-obvious step from a basic understanding of protein/protein and protein/DNA interactions to compounds that are complementary to a

targeted RNA molecule and inhibit the function of the targeted RNA molecule *via* binding to the targeted minor groove. This advancement (this is **not** an "improvement" *per se*) in the field of molecular biology has allowed subsequent research into the mechanism of RNA activity inhibition by providing the proper compounds a "route" of access into the potential complex RNA maze of potential Watson-Crick intrastrand hydrogen bonds between sequences and tertiary base pairing schemes that are typically non-Watson-Crick (such as Hoogstein pairing). The claims as pending offer a technological breakthrough in the field of RNA chemistry/molecular biology and relate directly to, what has befuddled researchers for years, inhibiting the activities of RNA molecules that assume a greater variety of tertiary structures than DNA molecules (see lines 34-6, bridging pages 2 and 3). Again, borrowing from the Supreme Court's first recognition of a pioneering patent (*Morley Sewing-Machine Co. v. Lancaster*, 129 U.S. 263, 1889), this is not a mere improvement upon a prior method or composition for the inhibition of RNA by binding a targeted minor groove, which is capable of accomplishing the same general result. The claimed invention clearly enlarges the field of the art. "The concept of the 'pioneer' arises from an ancient jurisprudence, reflecting judicial appreciation that a broad breakthrough invention merits a broader scope of equivalents that does a narrow improvement in a crowded technology." *Sun Studs, Inc. v. ATA Equipment Leasing, Inc.*, 872 F.2d 978, 987, 10 USPQ2d 1338, 1346 (Fed. Cir. 1989), modified, 872 F.2d 978, 11 USPQ2d 1479 (Fed. Cir. 1989). The Appellant submits that in view of the spectrum of prior art and novelty, the presently claimed invention falls within the category of very earliest of technological breakthroughs in the inventive field and is a distinct step in the progress of the art.

(vii) Summary

An understanding of nucleic acid structure is the result of a detailed analysis of the molecular interactions between proteins and their target nucleic acid (i.e. the hydrogen bonding arrangements and hydrophobic interactions between, for example, repressors and target sequence). One of skill in the art will recognize that, in view of the present specification, the identification of the critical region of the target RNA sequence dictates and defines the specific conformation and the "order" of complementary groups on the compound that must be assembled in order to recognize the target RNA region.

The specification shows at page 38, as pointed out by the Examiner, that the claimed genus of compounds which specifically inhibit the function of the targeted RNA are synthesized using methods known to those skilled in the art based upon the sequence and structure of the minor groove of the RNA. The specification also clearly describes the structure of the claimed compounds in view of the detailed description of the targeted minor groove of RNA that provides the geometric, spatial, hydrophobic, and hydrogen bonding constraints required for the claimed complementary compound to bind to a specific critical region and inhibit the RNA function.

As discussed in the declarations under 37 C.F.R. § 1.132, the geometric configuration of the target minor groove of RNA is predicated by the presence of hydrogen bonds, the hydrophobicity of the local environment, and "the repulsive and attractive forces that exist as electrostatic entities". Each of these target RNA attributes is described in the specification. For example, the extensive stacking and base pairing of planar aromatic purines and pyrimidines

inherently rendering the local environment inaccessible to solvent (or hydrophobic) is taught at page 2, lines 21-29. Furthermore, as disclosed at page 7, lines 24-26 (and again at pages 19 and 20), a network of hydrogen bonds provides not only for a stable structure within the minor groove, but also the establishment of a proper interface between compounds and their target nucleic acid (can be visualized as two pieces of a puzzle that fit together, wherein the properly spaced and oriented hydrogen bonds of the compound line the edge of one puzzle piece, and the accessible hydrogen bonds of the target RNA line the edge of the complementary piece). The maximization of these properties strengthens the electrostatic interaction between the compound and its target RNA. The end result is a specific (complementary) binding interaction that is dependent upon the defined compound structure as claimed and described.

It should be noted that Appellant is *not* claiming support for defining the structural features of the claimed compound based solely upon the functionality of "hybridizing" a compound to an RNA molecule. Appellant realizes that the Guidelines do not allow a compound be defined *only* by its function. Each *physical* characteristic of the target RNA molecule, as described in the specification and further supported by the declarations submitted herewith, contributes to the claimed complementarity of the inhibitory compounds, and therefore their structure. The formation of a complementary interface between the target RNA and the claimed compound is based upon, *inter alia*, the recognition of specific and accessible hydrogen bonds. This interface lies at the core of the presently claimed composition because it precisely defines the compound for which the target RNA will accept as its "partner".

While most, if not all, compounds that bind RNA do have hydrogen bonding sites, only a few will have the necessary pattern of sites to be utilized specifically by the targeted critical region. The definition of the critical region within the minor groove by a combination of primary, secondary and tertiary structure analysis, as recited in claim 11, determines the structure of the claimed compounds. Therefore, in view of the specification and the submitted declarations under 37 C.F.R. § 1.132, the structural features common to the claimed compound, as defined by the term "complementary" and by containing the requisite hydrogen bonding acceptor and donor sites, are clearly described.

In summary, the Appellant has not only described significant structural and physical properties of the claimed compound and its cognate RNA target, Appellants have additionally submitted two independent expert opinions that clearly support this assertion and describe the specification as being sufficient for one of ordinary skill in the art to realize the *structural* features that *define* the claimed compound.

(9) Summary and Conclusion

For the foregoing reasons, Appellant submits that the claims 11-13, 17-19 and 21 are patentable.

Respectfully submitted,

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Reg. No. 31,284

Date: September 10, 2002

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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Patrea Pabst

Date: September 10, 2002

Appendix: Claims On Appeal

Claims 1, 3-10, 14-16, and 20 are allowed.

11. A complementary compound comprising hydrogen bond donor and acceptor sites arranged to specifically bind and inhibit the function of a targeted RNA molecule, wherein the compound is specifically directed to and binds to a critical region of the RNA molecule, located within the minor groove of the RNA molecule, identified by a combination of the primary, secondary and tertiary structure of the critical region.

12. The complementary compound of claim 11 wherein the RNA is selected from the group consisting of mRNA, tRNA, rRNA, and viral RNA.

13. The complementary compound of claim 11 further comprising a pharmaceutically acceptable carrier selected from the group consisting of pharmaceutically acceptable compositions for topical administration, pharmaceutically acceptable compositions for parenteral administration, pharmaceutically acceptable compositions for enteral administration, and combinations thereof.

17. The complementary compound of claim 12 wherein the compound binds to a critical region within the minor groove of the acceptor stem of a tRNA molecule.

18. The complementary compound of claim 17 wherein the tRNA molecule is $tRNA^{Ala}$.

19. The complementary compound of claim 17 wherein the critical region is the G3:U70 base pair.

21. The complementary compound of claim 11 wherein the compound is a nucleic acid and the compound is synthesized *in vivo* from a retroviral vector.

TABLE OF CONTENTS

ATL1 #539552 v1

1: RNA 1995 Mar;1(1):7-20

Interactions between the double-stranded RNA binding motif and RNA: definition of the binding site for the interferon-induced protein kinase DAI (PKR) on adenovirus VA RNA.

Clarke PA, Mathews MB.

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.

The protein kinase DAI, the double-stranded RNA activated inhibitor of translation (also known as PKR), regulates cell growth, virus infection, and other processes. DAI represents a class of proteins containing a recently recognized RNA binding motif, the dsRBM, but little is known about the contacts between these proteins and their RNA ligands. In adenovirus-infected cells, DAI activation is prevented by VA RNAI, a highly structured RNA that binds to the kinase. VA RNA contains three chief structural features: a terminal stem, an apical stem-loop, and a complex central domain. We used enzymatic and chemical footprinting to identify the interactions between DAI and VA RNAI. DAI protects the proximal part of the apical stem structure, an adjacent region in the central domain, and a region surrounding a conserved stem in the central domain from nuclease attack. During binding the RNA undergoes a conformational change that is mainly restricted to the central domain. A similar change is induced by magnesium ions alone. Footprinting and interference binding assays using base-specific chemical probes suggest that the protein does not make major contacts with RNA bases. On the other hand, footprinting with probes specific for the RNA backbone shows that DAI engages in a strong interaction with the minor groove of the apical stem and a weaker interaction in the central domain. A truncated form of DAI, p20, containing only the RNA binding domain, gives a similar protection pattern in the apical stem but protects the central domain less effectively. We conclude that the RNA binding domain of DAI interacts directly with the apical stem and central domain of VA RNA, and that other regions of the protein contribute to interactions with the central domain.

PMID: 7489491 [PubMed - indexed for MEDLINE]

1: Biochemistry 2001 Feb 6;40(5):1150-8

Related Articles, Books, LinkOut

A heterocyclic inhibitor of the REV-RRE complex binds to RRE as a dimer.

Li K, Davis TM, Bailly C, Kumar A, Boykin DW, Wilson WD.

Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, USA.

As part of a search for organic compounds that selectively target RNA, we found that specific diphenylfuran derivatives, which are related to compounds that bind to the DNA minor groove, bind very strongly to RNA in a manner very sensitive to the structure of the compounds. In extended development of the diphenylfuran series, we found that a tetracationic heterocycle containing a phenyl-furan-benzimidazole unfused aromatic system, DB340, exhibits pronounced selectivity for the RRE RNA stem-loop from HIV-1. We report here RNA footprinting, spectroscopic analysis, affinity determinations, and initial NMR structural results of the complex. The results indicate that DB340 binds to RRE in a highly structured and cooperative complex at a 2:1 DB340 to RRE ratio. Overlap in the NMR spectra prevents detailed description of binding interactions at this time, but we are able to place DB340 in the RNA minor groove. Additionally, footprinting results and studies with mutant RRE sequences indicate that the internal loop of RRE is required for specific binding of DB340 as with the Rev protein. These results provide exciting new ideas for rational drug design with RNA as is now common with DNA and proteins.

PMID: 11170440 [PubMed - indexed for MEDLINE]

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1: Science 2000 Apr 7;288(5463):107-13 Related Articles, Nucleotide, Protein, Structure, Books, LinkOut

Full text article at www.sciencemag.org

Structure of the S15,S6,S18-rRNA complex: assembly of the 30S ribosome central domain.

Agalarov SC, Sridhar Prasad G, Funke PM, Stout CD, Williamson JR.

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The crystal structure of a 70-kilodalton ribonucleoprotein complex from the central domain of the Thermus thermophilus 30S ribosomal subunit was solved at 2.6 angstrom resolution. The complex consists of a 104-nucleotide RNA fragment composed of two three-helix junctions that lie at the end of a central helix, and the ribosomal proteins S15, S6, and S18. S15 binds the ribosomal RNA early in the assembly of the 30S ribosomal subunit, stabilizing a conformational reorganization of the two three-helix junctions that creates the RNA fold necessary for subsequent binding of S6 and S18. The structure of the complex demonstrates the central role of S15-induced reorganization of central domain RNA for the subsequent steps of ribosome assembly.

PMID: 10753109 [PubMed - indexed for MEDLINE]

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1: J Mol Biol 2001 Oct 12;313(1):35-48

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Central domain assembly: thermodynamics and kinetics of S6 and S18 binding to an S15-RNA complex.

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The 30 S ribosomal subunit assembles in vitro through the hierarchical binding of 21 ribosomal proteins to 16 S rRNA. The central domain of 16 S rRNA becomes the platform of the 30 S subunit upon binding of ribosomal proteins S6, S8, S11, S15, S18 and S21. The assembly of the platform is nucleated by binding of S15 to 16 S rRNA, followed by the cooperative binding of S6 and S18. The prior binding of S6 and S18 is required for binding of S11 and S21. We have studied the mechanism of the cooperative binding of S6 and S18 to the S15-rRNA complex by isothermal titration calorimetry and gel mobility shift assays with rRNA and proteins from the hyperthermophilic bacterium Aquifex aeolicus. S6 and S18 form a stable heterodimer in solution with an apparent dissociation constant of 8.7 nM at 40 degrees C. The S6:S18 heterodimer binds to the S15-rRNA complex with an equilibrium dissociation constant of 2.7 nM at 40 degrees C. Consistent with previous studies using rRNA and proteins from Escherichia coli, we observed no binding of S6 or S18 in the absence of the other protein or S15. The presence of S15 increases the affinity of S6:S18 for the RNA by at least four orders of magnitude. The kinetics of S6:S18 binding to the S15-rRNA complex are slow, with an apparent bimolecular rate constant of 8.0 x 10(4) M(-1) s(-1) and an apparent unimolecular dissociation rate of 1.6 x 10(-4) s(-1). These results, which are consistent with a model in which S6 and S18 bind as a heterodimer to the S15-rRNA complex, provide a mechanistic framework to describe the previously observed S15-mediated cooperative binding of S6 and S18 in the ordered assembly of a multi-protein ribonucleoprotein complex.

PMID: 11601845 [PubMed - indexed for MEDLINE]

1: J Mol Biol 2001 Nov 30;314(3):413-22

Related Articles, Books, LinkOut

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Interaction of the Bacillus stearothermophilus ribosomal protein S15 with its 5'-translational operator mRNA.

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The Bacillus stearothermophilus ribosomal protein S15 (BS15) binds both a three-helix junction in the central domain of 16 S ribosomal RNA and its cognate mRNA. Native gel mobility-shift assays show that BS15 interacts specifically and with high affinity to the 5'-untranslated region (5'-UTR) of this cognate mRNA with an apparent dissociation constant of 3(+/-0.3) nM. In order to localize the structural elements that are essential for BS15 recognition, a series of deletion mutants of the full cognate mRNA were prepared and tested in the same gel-shift assay. The minimal binding site for BS15 is a 50 nucleotide RNA showing a close secondary structure resemblance to the BS15 binding region from 16 S rRNA. There are two major structural motifs that must be maintained for high-affinity binding. The first being a purine-rich three-helix junction, and the second being an internal loop. The sequence identity of the internal loops differs greatly between the BS15 mRNA and rRNA sites, and this difference is correlated to discrimination between wild-type BS15 and a BS15(H45R) mutant. The association and dissociation kinetics measured for the 5'-UTR-BS15 interaction are quite slow, but are typical for a ribosomal protein-RNA interaction. The BS15 mRNA and 16 S rRNA binding sites share a common secondary structure yet have little sequence identity. The mRNA and rRNA may in fact present similar if not identical structural elements that confer BS15 recognition. Copyright 2001 Academic Press.

PMID: 11846555 [PubMed - indexed for MEDLINE]

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